THE INFLUENCE OF CERTAIN COLLOIDS UPON FERMENTATION.

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(Two Text-figures.)

[Read 27th April, 1927.]

Part iv. Agar Fibre in the Alcoholic Fermentation.

In Part ii of this series it was shown (These Proceedings, 1925, p. 345) that agar had a somewhat irregular action. It depressed and then accelerated the alcoholic fermentation of dextrose when present to the extent of 0·2%. With 0·1%, it slightly accelerated and with 0·05% the fermentation was depressed. Both in the fluid and in the solid state it markedly accelerated the fermentation of lactose by the high temperature organism, when present to from 0·3% to 0·6% (These Proceedings, 1924, p. 440). It had a strongly positive action when in the fibrous or the flocculent condition in the ammoniacal fermentation of urea (These Proceedings, 1925, p. 350) when present to the extent of 0·4%. In the lactic fermentation of dextrose, 0·2% had no action.

The small quantities of agar were used when it was added to the medium prior to sterilizing the fluids. It dissolved during the steaming and separated out in the flocculent state when the fluids cooled. Half of one per cent of agar which was the usual amount of the other colloids taken in the experiments caused the fluids to become very viscous and it did not seem that this condition was advisable for the alcoholic fermentation.

Small amounts were used in the following in which the agar had been treated with acid, washed and finally dried. In all the other experiments, the commercial fibre had been used.

Table i.—The Influence of Agar upon the Alcoholic Fermentations.
Percentage of Invert Sugar Consumed.

Days	2	3	4	5
No agar	17·5	40·8	63·7	79·4
Agar 0·05%	15·1	36·9	60·4	77·4
Agar 0·1%	15·1	36·9	62·9	81·8
Agar 0·2%	15·7	35·8	64·4	84·2

This bears out what had already been found with crude fibre, that small quantities (0·05%) depressed while those a little larger at first depressed and then accelerated (These Proceedings, 1925, p. 345, Table vi).

In all these experiments with agar and yeasts, the agar had been in the flocculent condition; it had been added to the nutritive fluids before sterilization. In the experiment about to be recorded, half of one per cent of agar was used and

in order to avoid a thickening of the media, the agar fibre was put into a dry tube and steamed upon several occasions after which it was dropped into the fluid just before seeding. The experiments with agar in the ammoniacal fermentation of urea showed that fibrous agar was almost as efficient as floccules of agar in accelerating the fermentation.

TABLE ii.—THE INFLUENCE OF AGAR FIBRE UPON THE ALCOHOLIC FERMENTATION.

Percentage of Invert Sugar Consumed.

Days	2	3	4	5
Control, no agar ..	16·6	38·6	60·1	77·6
Agar fibre, 0·5% ..	17·5	41·1	65·0	82·5

In this case the agar has shown a decided accelerating effect all through the fermentation and makes it appear that the previous indeterminate results were caused by the use of an insufficiency of the colloid.

PART V. OLD OR HEATED YEAST CELLS ARE NOT STIMULATED BY FULLER'S EARTH.

It was shown in an earlier paper (These PROCEEDINGS, 1923, pp. 48, 623) that the mineral and similar colloids were able to stimulate the cells of the high temperature organism after they had been weakened by chill. In this paper some tests were made to see how yeast cells that had been weakened by age would respond to the presence of one of the most active of the mineral colloids, namely, fuller's earth.

In the first the cells were twenty hours old, in the second the culture was twenty days old and the third was an old culture that had been kept at laboratory temperature for four and a half months. Films of the last showed long sausage shaped cells woven together into clumps as well as single cells. The clumps were broken up as much as possible by repeated passage through a capillary nozzle, a procedure employed in all cases where a homogeneous suspension is desired. The yeast was a stock Scotch distillery yeast and the three tests are grouped together for convenience, although they were made at intervals of three and of twenty days.

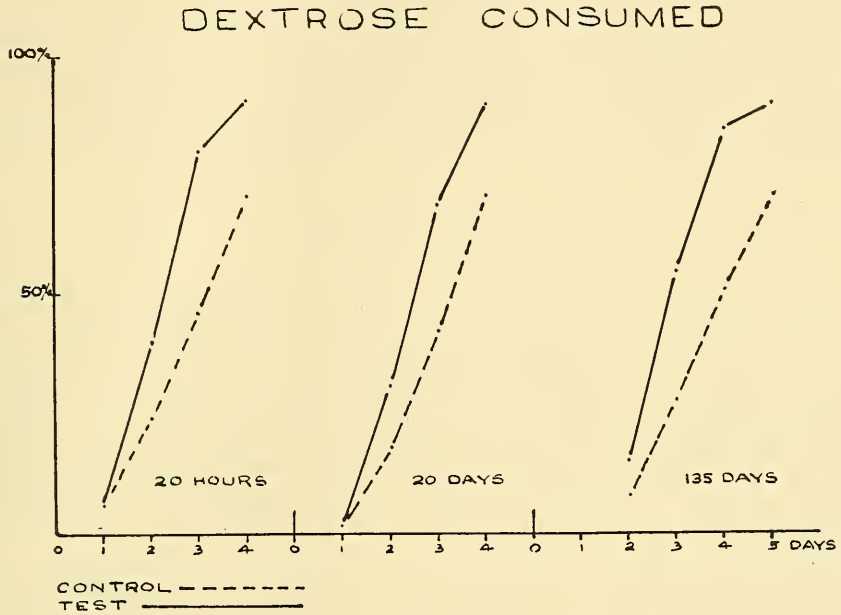
TABLE i.—THE INFLUENCE OF FULLER'S EARTH UPON THE FERMENTATION OF DEXTROSE BY YEAST CELLS OF DIFFERENT AGES.

Dextrose Consumed Per Cent.

Age of Cells.	Days	1	2	3	4	5
20 hours	Control	5·9	23·7	46·0	71·3	—
	Fuller's earth ..	6·7	39·9	79·9	90·6	—
20 days	Control	1·9	18·4	42·1	70·7	—
	Fuller's earth ..	1·7	31·3	68·7	90·0	—
135 days	Control	—	7·7	28·3	51·0	71·2
	Fuller's earth ..	—	15·2	55·2	84·8	90·5

The graphs of the three tests show that the younger cells began to ferment quicker but once fermentation had begun there was a similarity in the curves.

The mineral colloid did not show any differentiation between a young and an old culture of cells.



Upon finding that the colloid did not have an effect upon cells weakened by age and by contact with their own byproducts, an attempt was made to see if the application of heat would weaken them sufficiently to enable the colloid to exhibit an effect.

Suspensions of the same yeast were heated for half an hour at 45° and at 50° and sown in a dextrose medium which was incubated at 28° as in the previous cases. The following results were obtained; those for the unheated cells have already been recorded in Table i (20 hours).

Table ii.—The Influence of Fuller's Earth upon Heated Yeast Cells.

Percentage of Dextrose Consumed.

Days	1	2	3	4
Heated at 45°				
Control	5·9	24·7	48·2	70·1
Fuller's earth	5·6	41·3	81·1	90·5
Heated at 50°				
Control	none	10·0	32·7	60·7
Fuller's earth	none	13·2	49·7	82·9

Heating the yeast at 45° had no effect upon it, the dextrose consumed in the presence and absence of the mineral colloid was the same as by the unheated yeast (Table i, 20 hours). At 50°, it was weakened or partly destroyed and was slower to start the fermentation but the colloid did not accelerate the presumably weakened cells, indeed the differences between the control and the colloid determinations are less than with the unheated cells.

Heating the yeast cells therefore does not weaken them so much as to enable the colloid to exhibit a greater differentiation.

Conclusions.—The mineral colloid, fuller's earth, does not accelerate the fermentation of dextrose by yeast cells that have been weakened by age or by heat.

Part vi. The Non-adsorption of the Invertase of Heated Yeast by Fuller's Earth.

In Part v of this series, it was shown that the heating of yeast cells to 50° did not affect them to an extent to enable the mineral colloid, fuller's earth to show the stimulating effect which had been observed with weakened bacterial cells. Apparently the dextrose-fermenting function had not been affected. While this seems to apply to the alcoholic fermentation it might not apply to the other functions of the cell, for example, the secretion of invertase might be weakened and it might respond to the accelerating influence of a mineral colloid. With this idea in view, a number of experiments were made with yeast cells that had been heated to near the lethal temperature before being grown in solutions of saccharose.

The nutrient solution generally contained peptone 1 g., meat-extract 1 g., monopotassium phosphate 0·7 g., dipotassium phosphate 0·3 g., yeast-water 100 c.c., and magnesium sulphate anhyd. 0·3 g. in 500 c.c. of tap-water. The solution had a pH value of 6·8. To the above an equal volume of 16% saccharose solution was added generally just before seeding with the heated yeast. This solution of sugar had been filtered through porcelain to eliminate the spores of *Bac. levaniformans* which are always in sugar. The test flask or flasks received 0·5% of fuller's earth which had been sometimes ignited, treated with hydrochloric acid washed and dried or sometimes simply heated at 200°.

The first experiment gave a curious result and caused one to think that a novelty had been encountered but the succeeding tests were different and were more or less uniform. Five tests were made with heated yeast and five with normal unheated distillery yeast. All the experiments were made at different times and somewhat alternately, three heated, two normal, two heated and three normal but for convenience the results with the normal cells are tabulated first. In some of the tests the nutrient solutions were contained in small flasks and in these the fermentation proceeded rather briskly on account of the shallow layer of liquid formed by 50 c.c. The whole contents of these small flasks were taken for the determination of the sugars. In the majority of the tests, large flasks were used and portions of the 500 c.c. were daily abstracted for the determination of the reducing sugars before and after inversion at 70° with 10% hydrochloric acid. The reducing sugars were calculated to saccharose and expressed as percentages of the saccharose originally taken. The total inversion is the sum of the inverted saccharose present plus the saccharose lost, it being assumed that the saccharose

must have been first inverted before being fermented to alcohol, carbon dioxide and acids.[1]

In heating the yeasts the cells were suspended in a nutrient fluid generally a modified Hansen's fluid contained in Freudenreich flasks. These were immersed in water at 55° for varying times. After treatment, the flasks were incubated and that with the fewest colonies, as in *a*, was taken for seeding the test flasks. In *b*, the cells were suspended in a fluid of pH 6·2 and all flasks showed a good growth even when heated for 105 minutes; the last was distributed in a fluid of pH 5·6 and heated at 56°. This destroyed most of the cells, the 15-minute flasks contained two colonies which were grown, distributed and used. In *c*, the cells, suspended in a fluid of pH value 5·6, had been heated at 55° for 105 minutes. In *d*, the suspension medium had a pH value of 6·4 and the cells were heated for an hour at 54°. The growth that developed consisted of a large flocculent colony and many small ones; the small colonies were picked out, distributed and used. Similar colonies appeared as the result of heating a suspension in pH 5·7 for 50 minutes at 54°. The large flocculent colonies were picked out and used in *e*.

In the normal unheated set, 1 and 2 were made in small flasks and these show a rapid fermentation. The yeast in 5 was the crop from a single cell of the stock yeast which had been used in all the other tests.

The speed of the fermentation varied in the tests. This was partly due to differences in the amount of yeast added in seeding, partly to the amount of fluid in the fermentation flasks and possibly the amount of invert sugar originally in the fluids as the result of steaming the solution of saccharose might have had an influence. The invert sugar present at the start of each experiment was not always determined but in some cases it was. In *d*, there was 13%, *e* had 2%, 1 and 2 had less than 1·5%, 3 had none and 5 had 4%. Each pair of the test and control fluids was made at the same time with the same constituents, sown with the same number of drops of the same suspension of yeast, was contained in flasks of the same shape and capacity and was incubated side by side. One experiment may differ from another but each control can be compared with its test.

During fermentation, the pH values of the fluids fell, that is, the acidity rose from 6·8 to about 4·3 when from 80 to 90% of the sugar had been consumed. The control and the test were usually the same although in a few cases there was a difference of 0·1 or 0·2; the more fermented fluid was the more acid.

An examination of Tables i and ii and better, perhaps, of the graphs, shows that with the normal yeast the total inversion is slowed by the colloid, the consumption is slowed in four cases out of five; with the heated yeast, the total inversion was scarcely influenced by the colloid, the consumption was hastened in all cases.

It seems to be clear that normal yeast, if we may take this distillery yeast as a type, has its activity upon saccharose inhibited by the presence of a mineral colloid such as fuller's earth. This is probably the result of the adsorption of the yeast invertase by the colloid. Upon adsorption, the enzyme is rendered inert as has been shown by other workers and by the author (These PROCEEDINGS, 1925,

[1] In Part ii (1925) of this series the invert sugar as found has been given. It would have been better to have given the total inversion as this appears to be the proper way to express the enzymic activity. When the results of Table ii (of Part ii) are expressed in terms of the total inversion, it is shown that asbestos behaved like the fuller's earth in this paper (test *a*) and the loss of saccharose was much the same in the absence or presence of asbestos.

TABLE i.—THE ACTION OF NORMAL UNHEATED YEAST UPON SACCHAROSE IN PRESENCE OF FULLER'S EARTH.

In Terms of Original Saccharose.

Test	1		2		3			4			5			
Days	1	2	1	2	1	2	3	1	2	3	1	2	3	4
Control.														
Saccharose inverted ..	46	48	44	45	21	50	30	33	56	34	20	66	65	47
Saccharose consumed ..	16	47	13	54	24	42	69	6	34	62	3	15	31	52
Total inversion	62	95	57	99	45	92	99	39	90	96	23	81	96	99
Fuller's Earth.														
Saccharose inverted ..	32	56	36	44	15	42	37	21	42	27	17	60	70	60
Saccharose consumed ..	13	35	12	51	24	41	59	9	37	71	3	13	26	40
Total inversion	45	91	48	95	39	83	96	30	79	98	20	73	96	100

TABLE ii.—THE ACTION OF HEATED YEAST UPON SACCHAROSE IN PRESENCE OF FULLER'S EARTH.

In Terms of Original Saccharose.

Test	*a*			*b*			*c*			*d*			*e*		
Days	1	2	3	1	2	3	2	3	4	1	2	3	1	2	3
Control.															
Saccharose inverted ..	22	62	54	41	59	39	45	45	19	16	37	24	34	54	28
Saccharose consumed ..	4	20	44	11	32	60	30	52	80	9	40	72	15	36	70
Total inversion	26	82	98	52	91	99	75	97	99	25	77	96	49	90	98
Fuller's Earth.															
Saccharose inverted ..	22	62	55	43	52	32	45	36	10	15	34	21	29	44	19
Saccharose consumed ..	6	18	42	11	40	67	36	62	90	9	44	75	17	45	80
Total inversion	28	80	97	54	92	99	81	98	100	24	78	96	46	89	99

pp. 50, 342). The adsorption can be only partial as the production of reducing sugars is lessened and not prohibited. The slower production of fermentable hexoses has been followed by a slower alcoholic fermentation in four cases out of five, a result which seems to be quite natural. Had there been no slowing in the formation of the reducing sugars the colloid would have hastened the alcoholic fermentation, judged by the consumption of the sugar, as has been shown in Parts ii, iii and v of this series.

These experiments with heated yeast were made to see if heating the yeast to near the lethal temperature would influence the production of invertase and the results are in a positive direction. A change did occur in this particular yeast, grown in the particular medium. The invertase became indifferent to the presence of the mineral colloid, fuller's earth, and as a consequence the alcoholic fermentation of the inverted saccharose was accelerated by the mineral colloid. The practical outcome was that the heated yeast behaved towards saccharose just as an unheated yeast behaves towards dextrose or invert sugar in presence of the colloid.


TOTAL INVERSION
NORMAL YEAST
HEATED YEAST
DAYS
100%
50%
CONSUMPTION OF SUGAR
NORMAL YEAST
HEATED YEAST
CONTROL
TEST
60%
50%
DAYS

Conclusions.—Heating the yeast to near the lethal point led to a subsequent inversion of saccharose but the agent causing the inversion, presumably the invertase, was indifferent to the presence of the mineral colloid. The invertase of normal yeast is sensitive to the presence of the colloid, being partly adsorbed and producing a lessened inversion under similar conditions. As a result of the indifference, the alcoholic fermentation of saccharose by heated yeast is accelerated by the mineral colloids just as the fermentation of dextrose by a normal yeast is accelerated.